Specialty Conference

Brain Cellular Injury and Recovery—Horizons for Improving Medical Therapies in Stroke and Trauma

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An edited summary of an Interdepartmental Conference arranged by the Department of Medicine of the UCLA School of Medicine, Los Angeles. The Director of Conferences is William M. Pardridge, MD, Professor of Medicine.

After ischemic and traumatic brain injury, many cells may be rendered dysfunctional but are not irreversibly damaged or disrupted. The brain tissue may become metabolically deranged, and neurons, while still alive, are paralyzed and cannot create an action potential or conduct an electrical impulse. This injured brain tissue is in a precarious state of increased vulnerability. If the milieu of the cells is favorable, they may recover; if it is slightly unfavorable, they may die. There is now evidence that reversibly injured brain tissue will die from an ischemic or hypoxic insult ordinarily tolerated by the normal brain. The major challenge of modern research in stroke and trauma is to define the chemical and metabolic milieu in which the injured brain exists and to define an ideal milieu for healing.

(Becker DP, Verity MA, Povlishock J, et al: Brain cellular injury and recovery—Horizons for improving medical therapies in stroke and trauma [Specialty Conference]. West J Med 1988 Jun; 148:670-684)

DONALD P. BECKER, MD*: In the past decade there has been an explosion of new information on reversible brain cellular injury. This knowledge promises to provide medical therapies for improving the outcome in stroke and trauma previously unknown or considered impossible. Modern improvements in the treatment of patients with acute traumatic brain injury have significantly improved recovery and shown major reductions in morbidity and mortality. New information about metabolic brain tissue abnormalities, such as brain acidosis and the release of free radicals into brain tissue, provides more opportunities for improving treatment.

In human cases of acute brain injury—from mechanical trauma, ischemic or hypoxic stroke, spontaneous intracranial hemorrhage, compression from tumor or other mass—after the acute event, patients may improve and recover, or they may worsen and die. A new understanding of the mechanisms involved in the evolution of the processes and uncovering the critically involved mechanisms are providing important new insights that will lead to improved medical therapy.

That brain function can be temporarily impaired is an accepted premise. Consider the patient with a cerebral transient ischemic attack from embolus or focal hypoperfusion who has episodes lasting 30 seconds to several minutes of pronounced hemiparesis and then recovers completely. Or the patient who, after a generalized or focal seizure, has a Todd's paralysis that lasts up to 24 hours and then fully recovers. Although the mechanisms involved in these temporary losses of brain function have not been elucidated, they clearly represent a temporary, transient dysfunctional state in brain cells. Most of the tissue recovers completely.[1]

A more complex question relates to understanding the mechanisms of delayed or prolonged recovery after an acute neurologic insult. What happens to brain tissue in a patient who enters a hospital with brain trauma, is unconscious and unresponsive to his or her external or internal environment, and has unilateral decorticate posturing, who during the following weeks progressively awakens and in a matter of months shows major improvement in behavioral and neurologic functions? What factors are involved? The question is whether this represents the following:

- Progressive recovery of injured but not destroyed brain tissue, which lies relatively dormant in a dysfunctional but reversible state—based on the evidence presented later, this probably represents the major mechanism for recovery.
- Reorientation of brain circuitry or relations such that healthy brain tissue takes over the function of destroyed brain tissue—this occurs in young children who can transfer language function from an injured to an uninjured hemisphere; it has not been shown to occur in mature adults.
- Regenerative processes in brain tissue where damaged axons regrow to reestablish synaptic communication, or the ingrowth of uninjured axons occurs to replace vacated synaptic sites on empty dendritic zones—although central nervous system regeneration has been shown to occur in laboratory and clinical human studies, the functional usefulness of

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Supported by grants from the American Heart Association (Dr Cheung) and the National Institutes of Health grant NS-12587 (Dr Becker).
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ABBREVIATIONS USED IN TEXT

CK-BB = creatine kinase brain-specific [isoenzyme]
CSF = cerebrospinal fluid
EEG = electroencephalogram

these new connections is questioned. Recent nervous tissue transplantation studies in mammals suggest that new connections can become functionally useful.[2]

Increased Vulnerability of the Injured Brain

After an acute neurologic injury, the damaged brain tissue is more sensitive to a second injury than is a healthy brain. The damaged brain is operating with impaired metabolic machinery and cannot handle a second injury as well as normal tissue can. A simple experiment recently reported in cats proves this point.

After a mild concussive brain injury, which causes transient electroencephalographic (EEG) changes at impact, followed by a full EEG recovery in an hour and complete neurologic recovery of the animal at 24 hours, the cortex of the suprasylvian gyrus appears normal under light microscopy at 24 hours (Figure 1). After seven minutes of incomplete ischemia—95% reduction of brain blood flow—during which the EEG becomes isoelectric and then recovers several minutes after reperfusion, at sacrifice at 24 hours, the suprasylvian cortex again seems normal (Figure 2). When the normally recoverable insults of trauma and ischemia are administered sequentially, however—a reversible traumatic insult followed an hour later by the ischemic injury—the EEG, which recovers after the trauma, goes flat after the ischemic insult and does not recover with reperfusion of up to 24 hours. At sacrifice, the suprasylvian gyral cortex shows multiple dark, shrunken neurons indicating cellular death, accompanied by perineuronal and perivascular swelling (Figure 3).[3] Ishge and co-workers have recently shown the same kind of increased vulnerability to sequential hypoxic brain damage.[4]

From these observations, two important conclusions can be reached. First, injured brain tissue subjected to a second insult of ischemia or hypoxia, ordinarily well tolerated, may be devastated by this second injury. This is a clinically significant fact because second insults to an injured brain are a common occurrence, are correlated with a poorer patient outcome, and are potentially preventable. In a study by Miller and Becker,[5] 37% of patients suffering severe brain trauma had a partial pressure of arterial oxygen of less than 65 torr, and 15% were hypotensive, having a systolic arterial blood pressure of less than 90 torr (Table 1). A patient who suffers a moderate diffuse ischemic brain injury from arterial hypotension due to myocardial insufficiency may be devas-

TABLE 1.—*Systemic Insults Noted on Admission of 225 Consecutive Patients With Severe Brain Trauma**†*

Systemic Insult	*Patients, No.*	*%*	*Associated With MVA, %*	*Poor Outcome,‡ %*
Arterial hypoxemia—Po_2 < 60 torr	78	37	76	59§
Arterial hypotension—systolic BP < 90 torr	34	16	91	65§
Anemia—hematocrit < 30%	21	10	77	62
Arterial hypercarbia—Pco_2 > 45 torr	18	8	72	78§
None	117	52	73	35

BP = blood pressure, MVA = motor-vehicle accident, Pco_2 = partial carbon dioxide pressure, Po_2 = partial oxygen pressure

*From Miller and Becker.[5]
†Some patients had more than one insult.
‡Poor outcome includes severe disability, vegetative state, and death.
§Difference from the 35% "no insult" figure is significant, $P < .01$.

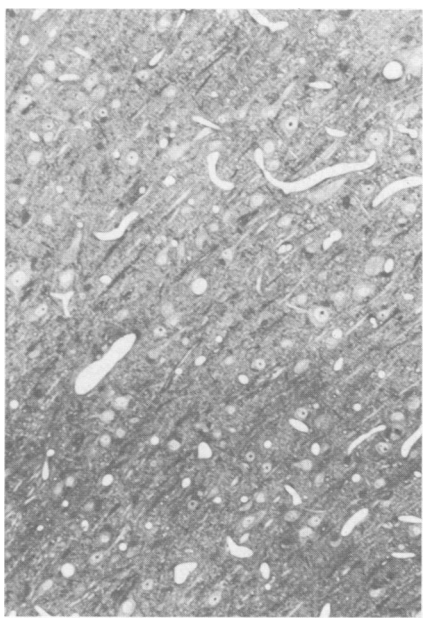

Figure 1.—The photomicrograph of cortex of the suprasylvian gyrus in a cat shows that animals subjected to moderate mechanical injury had normal structural cortex at 24 hours after injury (original magnification × 125).

Figure 2.—The photomicrograph of the same area as in Figure 1 shows that cats subjected to 95% reduction in cerebral blood flow had complete structural preservation of cortex at 24 hours after the ischemic insult (original magnification × 125).

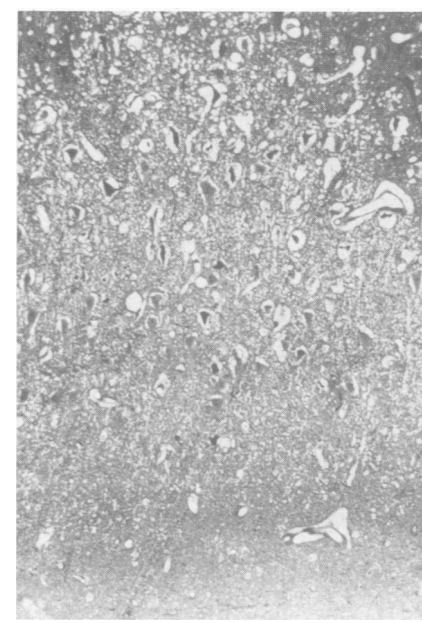

Figure 3.—The photomicrograph of a section of cortex of the suprasylvian gyrus in a cat shows that when normally reversible insults of trauma and ischemia are sequentially combined, severe structural changes are observed suggesting that even mild trauma renders the brain more vulnerable to ischemic insults (original magnification × 125).

tated by the delayed recognition of a second attack of hypotension, ordinarily reasonably tolerated.

The second important conclusion is that an injured brain, even one that suffers a moderate concussion or diffuse transient reversible ischemic insult, is in a metabolically abnormal state; the cells cannot handle the metabolic challenge of a second insult. Several laboratory and clinical studies recently conducted have begun to provide preliminary understanding of some of the abnormalities.

Acidosis and Lactate Accumulation in the Injured Brain

Cerebral acidosis occurs in humans after mechanical brain injury and during ischemic brain injury. Since Glaser first described increased levels of lactate in cerebrospinal fluid (CSF) in 1926,[6] several studies on CSF lactate accumulation after head injury have been reported, and most have assessed the prognostic value of CSF lactate levels.[7-15] High and progressively increasing lactate levels have been associated with a poor outcome. The lactate accumulation is intimately associated with the extent and location of brain injury. These changes in lactate concentration signify increased lactic acid production, which may be mainly responsible for the changes in brain pH and cerebrospinal fluid seen after injury.[16] And, indeed, the CSF pH characteristically falls after a brain injury, and the degree of fall is associated with the severity of injury.[17-19]

Cerebrospinal fluid is in communication with, and reflects changes in, brain extracellular fluid.[20(p44)] Extracellular edema fluid is thought to collect in the CSF by diffusion and bulk flow.[21-23] For lactate to appear in CSF, the clearance must be associated to some degree with the kinetics of fluid movement through brain tissue and the processes that govern flow from the brain into the CSF. Reulen and associates have shown that the clearance of edema by the ventricular CSF is a function of the ventricular pressure.[24] A low intracranial pressure would enhance fluid movement and hence the flow of lactate from brain to CSF. Marmarou and colleagues have reported that brain tissue is easily distended by the edema fluid and white matter must reach a level of saturation before fluid enters the ventricles.[25] These findings suggest that the clearance of lactate into CSF by the injured brain will be decreased when the intracranial pressure is elevated. Moreover, it would imply that because of the ability of the tissue to store the fluid, small changes in CSF pH may reflect large stores of tissue lactate.

Although blood in the spinal fluid will produce lactic acid, lactate elevations after brain injury primarily come from the brain itself.[18] In experiments, when blood is shed into the subarachnoid space, CSF lactate levels increase for five to six hours and then begin to fall off. With an acute intracerebral lesion, CSF lactate continues to rise and stays elevated after six hours.[26-28] In patients with a head injury, lactate levels may continue to rise even several days after injury in patients with more severe brain damage.[19]

The normal pH of the CSF is in the range of 7.32 ± 0.01 and is remarkably constant even in the presence of considerable systemic acid-base disturbances.[28-32] In cases of head injury, the pH of the CSF has been noted to drop as low as 7.269 ± 0.018 in patients in coma associated with decerebrate posturing and below 7.2 in patients with extensive injury, who ultimately die.[14,19] Even though the inherent buffering capacity of CSF is small when compared with blood, a high level of brain extracellular fluid acidosis can be expected to have occurred if the CSF pH is to be significantly lowered by a brain injury.[33,34] This is because the CSF reservoir is large (about 150 ml in humans) and the fluid is constantly being turned over and replaced at a rate of 500 ml per day. Patients with head injuries often have an arterial blood pH in the alkalotic range due to hyperventilation (spontaneous or iatrogenic). If the lowered pH of the CSF is due primarily to the addition of brain extracellular fluid, one can easily imagine extracellular fluid pH values well below 7.0. If ischemia is superimposed, the intracellular pH could theoretically fall to around 6.5 or below.[35-39] The exact level of intracellular and extracellular pH, which can lead to reversible or irreversible cellular injury, has yet to be determined.[40] Acid metabolites other than lactic acid may well accumulate after brain injury. Some experimental studies have shown a discrepancy between the excess CSF lactate accumulation and the associated CSF-bicarbonate reduction, suggesting the presence of additional, unidentified acid metabolites.[27,41]

Severe Cellular Acidosis From Brain Injury—A Cause of Additional Brain Damage?

Myers specifically advanced the hypothesis in 1979 that severe cellular acidosis from brain injury may be the chief cause of additional secondary or delayed brain damage.[42,43] In vitro studies in 1961 by Friede and Van Houten[44] and in vivo studies by Myers and Yamaguchi[45,46] indicated a deleterious effect on brain tissue of excessive tissue lactic acidosis. This led Myers to propose that the occurrence, degree, and distribution of brain injury resulting from hypoxia are determined by the accumulation of lactic acid in brain tissue. He presented evidence that when lactic acid accumulates at high concentrations—greater than 20 μmol per gram of tissue—tissue changes develop that lead to altered cell membrane structure and function, to a breakdown of the blood-brain barrier, to brain edema, and to widespread injury to brain tissue.[42] Lactic acid accumulation is quantitatively related to the carbohydrate concentration in the brain during a hypoxic or ischemic insult. The influence of increased blood and brain glucose levels on the degree of brain injury was discovered by Myers and Yamaguchi[45,46] and has been confirmed since then in studies by Pulsinelli and Petito[40] and Diemer and Siemkowicz.[47]

Studies by Rehncrona and associates[38] and Kalimo and co-workers[49] have established the important influence of severe brain lactic acidosis in the ischemic brain and its detrimental effect on postischemic recovery.[36-38,48] Increased lactic acidosis occurred in animals with hyperglycemia; by varying tissue lactate levels, recovery of the energy state and neurophysiologic variables was not only altered, but the degree of impairment was related to the level of lactate accumulation. Because lactic acid production is associated with hydrogen ion generation, a likely mechanism of the loss of neurophysiologic function and impairment of the energy state recovery is deranged intracellular pH homeostasis. This derangement may seriously affect many reactions and enzyme systems, the integrity of which is necessary for cellular viability.

The effect of lactic acidosis on normal versus abnormal cells—where lactic acidosis accumulates because of the abnormality of the biochemical machinery—may be quite different; for example, the insult survived by healthy tissue may be fatal in damaged tissue. Another factor is that normal

tissue has mechanisms that limit lactic acid production as the pH declines. These feedback mechanisms may not be functional in damaged cells.

Yang and colleagues have recently reported the accumulation of lactate in brain tissue after only moderate brain injury (Figure 4).[50] They postulated that the lactate accumulation is due to impaired mitochondrial function where aerobic metabolism normally takes place. The injured cells may be metabolizing glucose anaerobically, producing less high-energy phosphate, which could partly explain the increased vulnerability state. DeSalles and co-workers recently reported that lactate accumulation continues in the brain-spinal fluid for days after severe brain injury.[51] The level of lactate in cerebrospinal fluid correlates with the outcome (Figure 5).[6]

Rabow and associates recently studied the posttraumatic creatine kinase brain-specific isoenzyme (CK-BB isoenzyme) and lactate concentration activity in cerebrospinal fluid in 29 patients with severe head injuries.[52] The CK-BB isoenzyme activity reached its maximum a few hours after trauma and had a monoexponential drop with a half-life (τ½) of about ten hours, whereas the CSF lactate concentration continued to rise in patients with a poor outcome and decreased only slowly and inconsistently in most of the other patients. This, together with the fact that intracranial pressure problems in the posttraumatic course showed no correlation with CSF lactate levels during the first 24 hours after trauma but were significantly correlated with maximum CK-BB activity, indicates that lactate increase in the cerebrospinal fluid is not, like the CK-BB isoenzyme, a direct, one-stage consequence of the trauma but is due to a continuous production from a derangement of metabolism caused by the trauma. Because even higher CSF lactate levels were survived when not caused by the head injury, and because no significant pH changes were related to the CSF lactic acidosis in these patients when they were ventilated, CSF lactic acidosis is therefore indicative of a severe, although not necessarily intractable, disturbance of brain function associated with intracellular lactate production and acidosis.

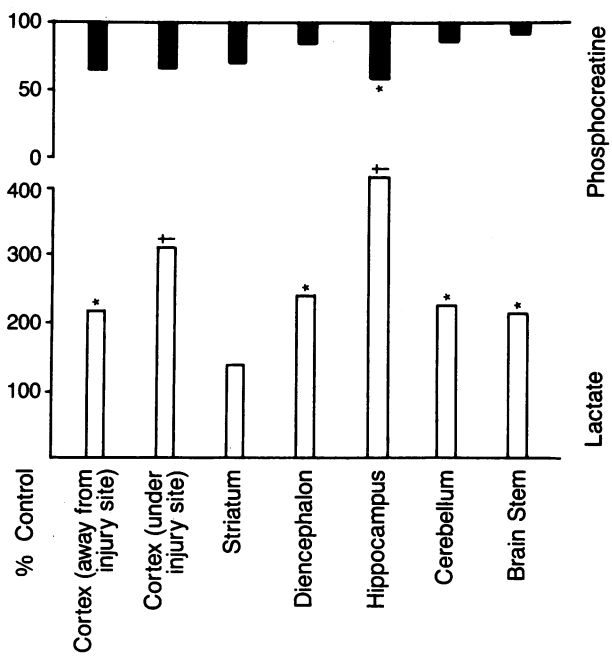

Figure 4.—The graphs show the percentage changes in lactate and phosphocreatine levels from control values noted in uninjured control cats calculated for different brain regions 1 hour after a 2.0-atm fluid-percussion injury. Statistically significant differences are $*P<.005$; $\dagger P<.01$.

Arachidonic Acid Release, Prostaglandin Synthesis, and Release of Oxygen Free Radicals in Brain Injury

Ellis and colleagues have shown the release of arachidonic acid after a brain injury.[53] The brain makes both lipoxygenase and cyclooxygenase enzyme products from arachidonic acid. Free radicals are released during the cascade that can cause anatomic damage to cerebrovascular endothelium and impair normal carbon dioxide reactivity (Figure 6). The damage to CO_2 reactivity can be prevented by prostaglandin synthesis inhibitors and administering free radical scavengers, such as superoxide dismutase, nitroblue tetrazolium, or mannitol (Figures 7 and 8).

Kontos and co-workers showed the appearance of superoxide anion radicals in the cerebral extracellular space

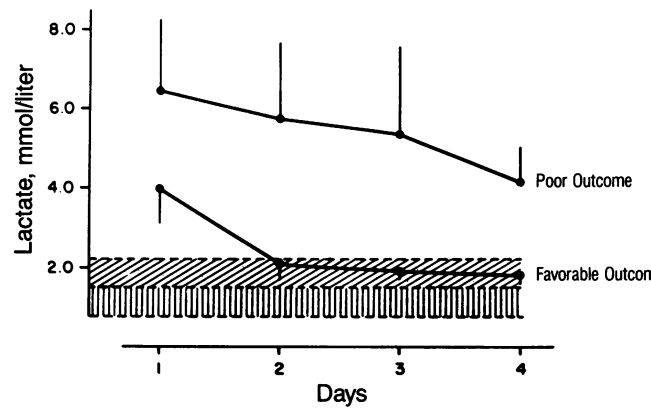

Figure 5.—Lactate levels in cerebrospinal fluid in patients with severe brain injury may remain elevated for days after the injury. This is indicative of continuous lactate production in brain. High lactate levels are associated with a poor outcome.

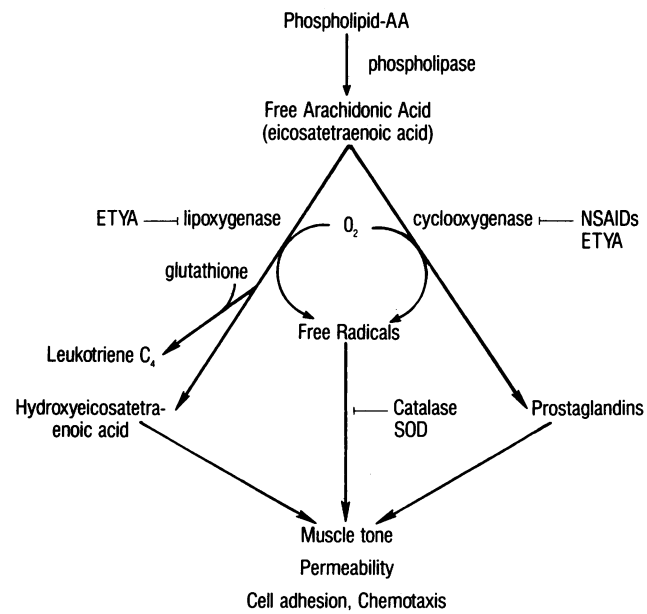

Figure 6.—In cases of brain injury, arachidonic acid is released. This results in a chain of metabolic reactions that result in the production of oxygen free radicals. ETYA = eicosatetraynoic acid, NSAIDs = nonsteroidal anti-inflammatory drugs, SOD = superoxide dismutase

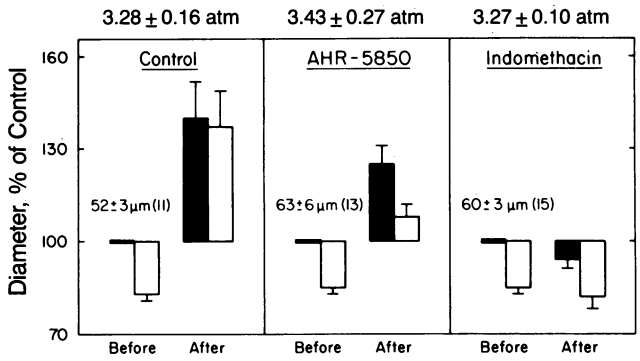

Figure 7.—The graphs show cat cortical arteriolar diameters viewed through a cranial window. With traumatic injury, arterioles dilate, associated with loss of carbon dioxide (control experiment). Prostaglandin synthesis inhibitors (AHR-5850 and indomethacin) given before the injury preserve CO_2 reactivity. The numbers in parentheses are the number of subjects. ■ = normocapnia, □ = hypocapnia

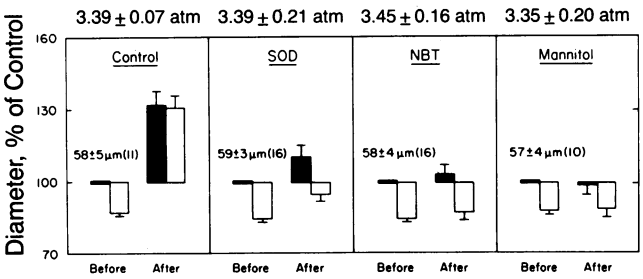

Figure 8.—The graphs again show cats' cortical arteriolar diameters. In this experiment, similar to that in Figure 7, pretreatment with free radical scavengers—superoxide dismutase (SOD), nitroblue tetrazolium (NBT), and mannitol—preserves carbon dioxide responsiveness following percussion injury. The numbers in parentheses are the number of subjects. ■ = normocapnia, □ = hypocapnia

during increased prostaglandin synthesis in cats.[54] When increased prostaglandin synthesis was induced in anesthetized cats equipped with cranial windows by the topical application of arachidonate (200 μg per ml) or bradykinin (20 μg per ml), there was a reduction of nitroblue tetrazolium, resulting in a deposition of the reduced insoluble form of this dye on the brain surface. The amount of reduced nitroblue tetrazolium was measured spectrophotometrically after the brain was fixated by perfusing with aldehydes to eliminate interference from hemoglobin. The topical application of 56 units per ml of superoxide dismutase or 20 μg per ml of indomethacin inhibited nitroblue tetrazolium reduction by 76.5% to 82.5% and 78% to 85.5%, respectively. These results show that most of the nitroblue tetrazolium reduction was accounted for by a superoxide anion radical generated in the course of arachidonate metabolism through the cyclooxygenase pathway. No superoxide production could be detected in the absence of arachidonate or bradykinin. Histologic examination showed no evidence of parenchymal cellular damage or vascular damage and no accumulation of leukocytes. Pronounced leukocyte accumulation occurred 24 hours after topical arachidonate application in rabbits with permanently implanted cranial windows. The superoxide appearance was reduced severely by administering 4,4′-diisothiocyano-2,2′-stilbene disulfonate and phenylglyoxal, two specific inhibitors of the anion channel. The most likely explanation for these findings is that increased metabolism of exogenous or endogenous arachidonate through cyclooxygenase results in the appearance of a superoxide anion radical in the cerebral extracellular space. Superoxide crosses the membrane of undamaged cells by the anion channel.[55]

Release of the superoxide anion radical and other yet-to-be-defined toxins into the extracellular space by partially damaged tissue, or from blood or disrupted brain tissue, could permanently damage cells ordinarily destined for re-

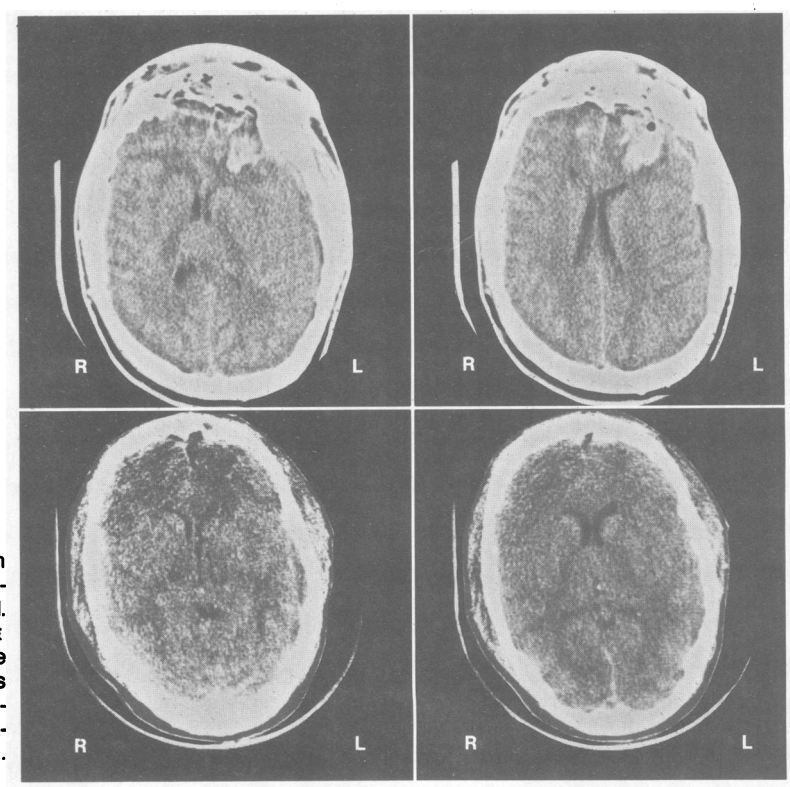

Figure 9.—**Top,** Computed tomographic scans were taken of a 19-year-old UCLA football player who sustained a bifrontal skull fracture and hemorrhagic contusion after a fall. The patient was confused with intermittent lethargy but without focal deficit. He underwent craniotomy with complete resection of hematoma and contused tissue. **Bottom,** Scans taken 5 days postoperatively show mild edema but no evidence of hematoma or hemorrhagic tissue. Clinically the patient did well, returning to school 6 weeks after the operation. The only deficit was anosmia.

covery. This delayed chemical injury may explain the progressive worsening sometimes seen in patients with acute brain injury, even in the absence of impaired cerebral blood flow, an elevated intracranial pressure, or inadequate delivery of nutrients to brain tissue. Even small volumes of blood accumulated in brain tissue may cause diffuse changes in tissue metabolism and a diffuse alteration in the EEG. That blood itself releases chemicals probably damaging to surrounding tissue is a popular hypothesis now being studied in several laboratories.

Two recent cases help explain the point: In one patient who entered hospital with a severe diffuse brain injury, a frontal lobe contusion was removed in two hours after the injury. At five days his computed tomographic scan looked almost normal and he made an excellent recovery (Figure 9). Another young patient was only dazed after falling off a golf cart and striking his head. His frontal lobe contusions were not removed, and at 24 hours after the injury he had gone from sleepy but alert to comatose and decerebrate; the lesions had enlarged remarkably (Figure 10).

The most exciting aspect of the recent discovery of a partial cellular injury associated with metabolic dysfunction is that it promises the early development of new modes of medical treatment. Morbidity and mortality after stroke and trauma can be significantly improved. For example, Newlon and co-workers have shown that half of patients who arrive alive at a hospital after a severe brain trauma die despite their having initial brain electrical activity that is consistent with their making a good recovery.[56] Improved management methods are sorely needed for such patients, as well as those with acute stroke. Dealing directly to reverse biochemical abnormalities and improve metabolic dysfunction may be an important direction of future therapy to improve brain tissue recovery.

TABLE 2.—*Neuropathologic Classifications of Head Injury*

Primary or impact damage	Secondary or complication injury
Cerebral contusions	Diffuse white matter degeneration*
Intracranial hemorrhage	Epilepsy
Diffuse axonal injury	Progressive neurologic disease
Hypoxic brain damage	Substantia nigra depigmentation
Edema	Neurofibrillary tangles
Fat embolism	Concussion

*From Strich.[59]

Cerebral Concussion as Head Trauma—Morphobiochemical Aspects

M. ANTHONY VERITY, MD*: Brain damage resulting from head injury has major socioeconomic effects on society. Significant intellectual, psychological, or personality changes impose financial and mental strains on friends and family.[57] In this conference we shall consider the various forms of brain damage induced by trauma and especially by concussive injury.

Classifications of brain damage resulting from nonmissile-induced head injury encompass clinical pathologic or pathogenetic mechanisms, or both. Unfortunately, the overlaps between various forms of brain injury with the merging of primary and secondary types of injury make it difficult to propose a clear-cut, meaningful classification. We have found a classification based on primary (or impact damage) and secondary (or complication injury) effects to be of value. Primary or impact damage includes both focal and diffuse brain injury. In this classification (Table 2), concussive injury represents a separate clinicopathologic problem.

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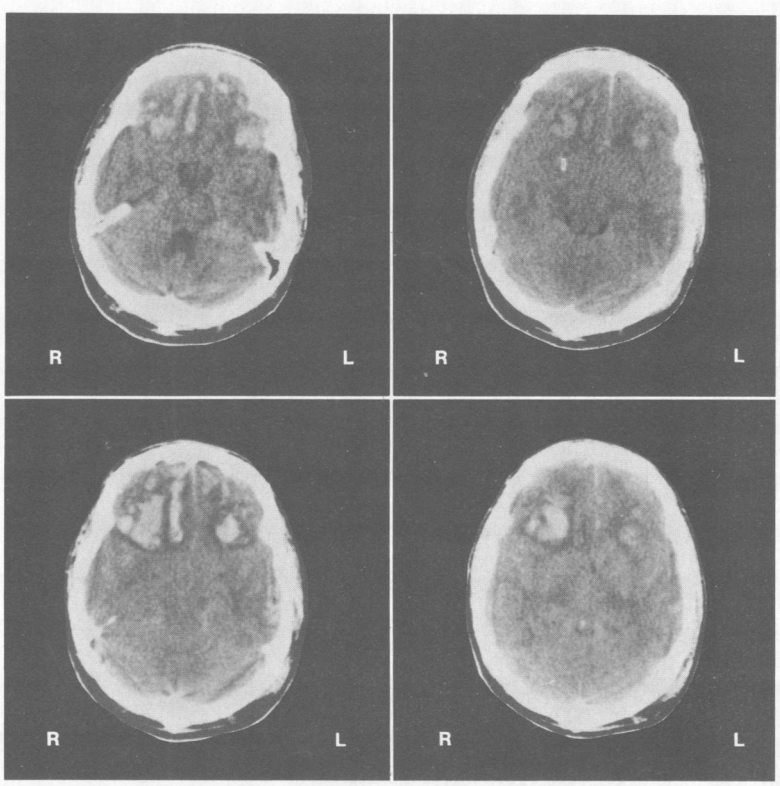

Figure 10.—Top, Computed tomographic (CT) scans were taken of a 23-year-old man struck in the occipital region with resultant bilateral contrecoup lesions. On presentation, the patient was conversant but had deteriorated within 36 hours, becoming obtunded and posturing intermittently. **Bottom,** The CT scans show consolidation and enlargement of hemorrhagic tissue. Management elsewhere included hyperventilation and the administration of steroids without surgery. Shortly after the second scan, the patient had herniation of his brain and died.

Cerebral contusions are punctate or focally confluent hemorrhages, usually at right angles to the cortical surface with emphasis at the crown of the gyrus. These may resolve with appropriate scarring of the gyri, shrinkage, and hemosiderosis; their distribution in the brain is characteristic. The orbital surfaces of the frontal poles, temporal poles, and cortex above and below the sylvian fissure are involved. Intracranial hemorrhage may be extradural, subdural, or intracerebral with variable overlap. The intracerebral hematomas of head injury are often multiple and occur deep within the hemispheres. They result from direct rupture of intrinsic cerebral vessels and may be predicated on preexisting intrinsic vascular disease, especially those associated with accelerated cerebrovascular atherosclerosis or with congophilic angiopathy accompanying presenile dementia.[58]

Since the original description by Strich,[59] the concept of diffuse axonal injury has received strong support from the detailed clinicopathologic studies undertaken by Adams and associates.[60,61] Such diffuse axonal damage is usually identified in the corpus callosum, the rostral brain stem, or as diffuse change throughout the brain. These findings are manifested neuropathologically in the classical retraction balls of Cajal shown in silver-impregnated preparations. In chronic disorders, clusters of microglia throughout the white matter are evident, often associated with wallerian-type degeneration at a distance. Povlishock and colleagues identified axonal retraction balls in cats subjected to minor head injury and found that these changes are not due to axonal rupture but represent a segmental axonopathy followed by segmentation, rupture, and retraction.[62]

The problems of hypoxic brain damage and edema have long been recognized[63] and are often represented as boundary zone hypoxic-ischemic lesions in regions between the major cerebral arterial territories. Arterial spasm is now accepted as occurring after head injury.[64] The pathogenesis of brain swelling, although a major factor in contributing to the raised intracranial pressure, is unclear. Swelling adjacent to contusions may be expected, but diffuse bilateral cerebral hemisphere swelling must relate to increased permeability at a capillary level with loss of physiologic regulation. Povlishock and co-workers found that horseradish peroxidase migrated across the capillary endothelium as a result of increased pinocytosis.[65]

We shall present the morphologic and presumed metabolic basis for concussive brain injury. Since the early investigations of Windle and Groat and colleagues, the term has come to imply a traumatic disruption of the functional integrity of the nervous system with sudden loss of consciousness.[66,67] The role of the brain-stem reticular activating system has been analyzed[68]; this portion of the brain stem is significantly affected, although the structural, biochemical, and electrophysiologic correlations are still not clear. Various pathogenetic mechanisms have been proposed (Table 3).[62,66,69-72] Retrograde degenerative changes have been identified in the neurons of the brain stem after concussion.[66,72,73] The role of the cervical spinal cord in inducing such retrograde neuronal degeneration is still unclear. Various conflicting studies have examined the permeability of brain blood vessels after experimental head injury, especially concussions. Many of these early studies failed to detect changes in vascular permeability.[74,75] More recent studies, however, have shown significant early changes in vascular permeability preferentially located in the brain stem and upper cervical cord, probably accounting for the failure of detection by other investigators.[69,70] Hayes and associates have recently collected data pertaining to the apparent selective involvement of the brain stem and pontomesencephalic area after concussive injury.[71] Using the method of Sokoloff and colleagues,[76] these investigators found that cerebral concussion in the rat promoted reversible behavioral suppression associated with an increased rate of glucose use in the dorsomedial pontine tegmentum. Such increased local glucose use represents a functional activation of this brain-stem region. These recent data are of interest because of the findings by Brown and co-workers, who showed ultrastructural and biochemical evidence for glycogen dysmetabolism in the brain-stem reticular formation after controlled concussive injury in guinea pigs.[72] The polysaccharide accumulated in dendrites, axon terminals, and in variable amounts in astrocyte cytoplasm.

The pathogenesis of such polysaccharide accumulation is unclear, and significant questions remain. What changes in metabolic pathways may be activated to account for the increased accumulation of glycogen? Why does glycogen preferentially accumulate in astroglia in local brain injury? The functional significance of nervous tissue glycogen is still unclear, but it probably serves as an energy reserve for neurons. The predominantly glial localization with an intimate relation of glial processes between neurons and the microvasculature provides a storage reserve capable of being altered in pathologic and disease states.

A key to understanding the metabolic interactions between glial cells and neurons rests in the signals by which such interactions may be controlled. Accumulating evidence highlights the role of potassium in this respect (Table 4).[77-79] Kai-Kai and Pentreath and colleagues have shown a more precise event evoked by the potassium signal[80,81]; they used the finding that radioactively labeled 2-deoxyglucose is selectively incorporated into glycogen, which unlike glucose effectively remains trapped within the glycogen. The distribution of labeled 2-deoxyglycogen can be studied by electron

TABLE 3.—*The Pathogenesis of Concussive Injury*

Diffuse axonal injury*
Brain-stem neuron chromatolysis†
Increased vascular permeability with exudation—lateral brain stem‡
Blood-brain barrier increases permeability to PO_4^{-3}§
Cerebral glucose use decline in midbrain (except pontine tegmentum)‖
Glycogen accumulation in brain stem¶

*Povlishock et al.[62] §Cassen and Neff.[70]
†Windle et al.[66] ‖Hayes et al.[71]
‡Rinder and Olsson.[69] ¶Brown et al.[72]

TABLE 4.—*Proposed Significance of Extracellular Potassium Concentration in Astrocyte Metabolism*

Restores K^+ levels
Restores other ions (not by activating sodium-potassium pump)
Redistributes K^+
Glial metabolism (tissue culture)
Increased O_2 consumption
Increased glucose uptake
Pyruvate kinase activation
2-Deoxyglucose experiments*

*See text for description.

microscopy. These investigators found that elevated extracellular potassium levels produced an increased incorporation of 2-deoxyglucose into glycogen of glial cells surrounding activated neurons. This study shows that increased neuronal activity causes a potassium-mediated increase in 2-deoxyglucose uptake and incorporation into the glycogen of glial cells. The role of potassium in controlling glycogen metabolism is well established, but it is not known which enzymic loci are potassium dependent. We can thus develop a hypothesis (Figure 11) that after concussive injury, pathologic concentrations of potassium accumulate in the interneuronal-glial interspace, especially around axodendritic and synaptic regions. In this model, the adjacent glial cells see a potassium signal, which stimulates glycogenesis within the glial cell and adjacent synaptic region.

These observations on morphologic and biochemical expressions after a concussive brain injury suggest a mechanism whereby the neuron-glial cell metabolic interaction can be disturbed. We have seen how an abnormal potassium signal may influence glycogen metabolism. The possibility of other interactions relating to a disordered trophic role or abnormal depolarization may be postulated. There is no doubt that the ultimate effect of single or multiple concussive episodes will be to impair the established interactions between neurons and glia. An analysis of such cell-cell interaction will be of value in understanding the pathogenesis of concussive injury and possibly account for the well-recognized postconcussive syndromes of amnesia and dementia.

Axons Injured in Brain Trauma

JOHN POVLISHOCK, PHD*: In the preceding passages, considerable attention has been devoted to the pathophysiology of brain injury, showing cellular, metabolic, and functional changes subsequent to the traumatic episode. The role of axonal damage in dictating the outcome and neurologic state in those patients whose traumatic course has been uncomplicated by mass lesions or secondary insults has been considered. In this context, then, one can appreciate that axonal injury is an important component of head injury and worthy of detailed investigation. Traditionally, axonal injury has been studied by postmortem examination of head-injured humans in whom the presence of enlarged reactive axonal swellings, classically characterized as retraction balls, was considered evidence of traumatically induced axonal damage.[61,82] Such axonal injury has been described in cases of severe, moderate, and even minor head injury, and it was thought that the shear and tensile forces of a traumatic event physically tore axons.[61,82,83] This event caused immediate axonal retraction with the expulsion of an axoplasmic mass forming an enlarged reactive swelling, the retraction ball.[61,82] As this concept of axonal injury implied that head injury elicited an immediate and devastating form of damage resulting in overt axonal discontinuity, the overall inference of this finding was rather grim. Regardless of the skill of a clinician or rigor of the therapeutic approaches, it seemed that nothing could be done to either stabilize or reverse that axonal damage elicited by the immediate, traumatically induced tearing of axons.

In more contemporary studies of head injury using well-controlled animal models of injury, these traditionally held concepts have been rejected, which has necessitated a reevaluation of axonal injury and its clinical ramifications. In our laboratory, we have systematically studied the posttraumatic evolution of reactive axonal change in cats subjected to minor or moderate forms of fluid-percussion brain injury.[62] By assessing the anterograde axonal transport of horseradish peroxidase studied at the light and electron-microscopic levels in these cats, we have found no evidence to support the concept that head injury elicits immediate axonal tearing with reaction ball formation.[62] Rather, we recognized that the compressive or tensile forces, or both, associated with the traumatic episode triggered more subtle yet progressive forms of change. Immediately after trauma, those long tract systems assessed showed no overt disruption; rather, discrete focal abnormalities were seen. Focal axolemmal irregularity was first recognized and, during a three-hour posttraumatic course, this focal change was associated with microtubular clumping and initial organelle pooling, which was linked to an impairment of axoplasmic transport. With continued survival, this focal organelle accumulation progressed; it was associated with axonal lobulation and with the formation of axonal swellings connected by a thinned axonal segment. Ultimately, these swellings separated and thereby caused overt axonal discontinuity. The overlying myelin sheath, when present, then underwent focal separation, collapsing to coalesce and encompass the now-detached proximal and distal axonal swelling. During the next 12 to 24 hours, the proximal swelling increased further in mass and in organelle content because of the impaired axoplasmic transport, resulting in the formation of a reactive swelling (retraction ball) of classical description. The distal detached segment, on the other hand, showed the onset of wallerian degeneration.

These observations made in minor-to-moderate experimental head injuries are of interest from several perspectives. From the clinical aspect, the delayed posttraumatic

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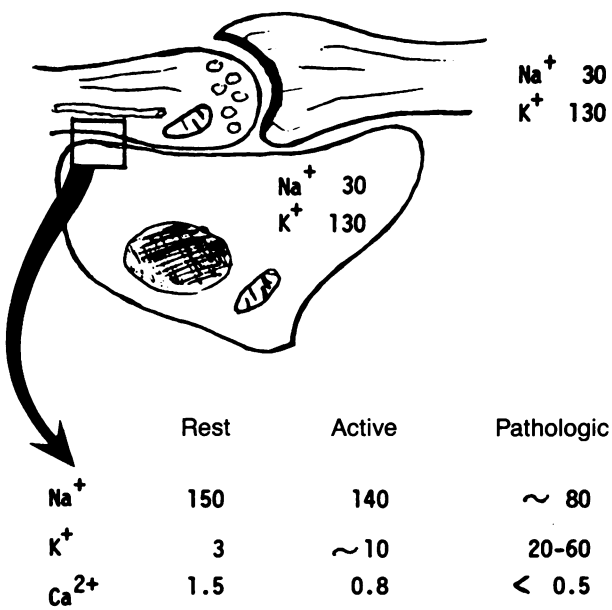

Figure 11.—The values for extracellular concentrations (millimoles per liter) of potassium (K^+), sodium (Na^+), and calcium (Ca^{2+})—represented in the neuronal-glial interspace—are given for the resting or active state and after massive depolarization, for example, postconcussive state. The abnormal values for potassium are sufficient to stimulate glial glycogenesis.

development of axonal separation with eventual reactive swelling implies that there is a time frame after head injury during which potential therapeutic approaches may be capable of either stabilizing or reversing the progress of the above-described axonal changes. Moreover, from the neurobiologic perspective, the subtle progressive temporal development of the reactive axonal swellings poses many questions in regards to their long-term fate. This is of particular interest because such reactive axonal swellings frequently occur in otherwise unaltered brain tissue. The related neuronal somata and dendrites show no abnormality,[84] and comparably the related vasculature manifests no mechanical disruption, alteration in blood-brain barrier status, or reduced regional blood flow.[65,85] Thus, given the relative intactness of the related brain parenchymal microenvironment, one would naturally question whether the traumatically induced swellings would, over time, remain unchanged, degenerate, or mount a regenerative attempt.

To explore this issue, we began to study the fate of traumatically induced reactive axonal swellings over a three-month posttraumatic course.[85,86] Cats were anesthetized and subjected to a fluid-percussion injury.[87] Essentially, such an injury entailed delivering a hydraulic pressure transient to the surface of the intact dura overlying the superior sagittal sinus. The hydraulic pressure caused an elastic deformation of the underlying brain, and the severity of the injury, determined by a transducer, was permitted to range from 1.3 to 2.5 atmospheres during an 18-millisecond period. Such injuries were in the minor-to-moderate range, which resulted in a transient concussive response. After the injury, the animals were permitted to recover and then were evaluated at various time points during a three-month period. Two days before the end of the desired survival period, the animals were reanesthetized and their major cerebral cortical and cerebellar efferents were anterogradely labeled by means of cerebral and cerebellar implants of wheat-germ agglutinin conjugated to horseradish peroxidase gels.[88] We used anterograde peroxidase passage because our previous studies showed this approach most useful in labeling reactive axonal swellings.[62] At the designated period of survival, the animals were anesthetized and perfused with aldehydes. Their brains were removed and sectioned on a vibratome, with adjacent sections being processed for either light-microscopic[89] or electron-microscopic[90] visualization of the peroxidase reaction product within those reactive swellings under investigation.

Within the first week after injury, all animals showed reactive axonal swellings localized within the cerebral peduncles, the brachium conjunctivum, the basilar pons, the red and vestibular nuclei, and the reticular core. Most of these swellings appeared within the first 24 hours after injury and, as such, appeared as organelle-laden masses capping a neurofilamentous core (Figures 12 and 13). Although such relatively unchanged reactive swellings predominated during the first week after trauma, some showed signs of the onset of degenerative change, whereas others showed the initiation of a regenerative attempt (Figure 12). Specifically, some of the swellings showed complex lobulation with neurofilamentous hyperplasia or increased electron density, all of which seemed consistent with retrogressive change. Other swellings, however, showed numerous reactive sprouts that emerged from the swelling proper (Figure 12). The sprouts ranged from 0.5 to 2 microns in diameter and up to 20 microns in length. They contained tubular and vesicular profiles of smooth endoplasmic reticulum and dense core vesicles. Concomitant with the appearance of the sprouts, the reactive swelling proper showed a reduction in size, most likely due to the redirection of its volume into the numerous sprouting neuritic outgrowths.

With continued posttraumatic survival, the above-described variable axonal responses were studied further. By the end of the first month after trauma, unchanged, degenera-

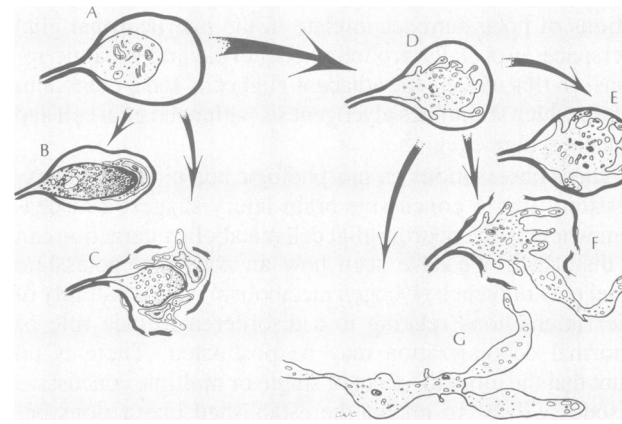

Figure 12.—The diagram shows reactive axonal changes that typically occur within the first month after head injury. Traumatically induced reactive axonal swellings (**A**) can either undergo degenerative change (**B** and **C**) or give rise to a regenerative response, characterized by sprouting (**D** and **E**) and growth cone-like outgrowths (**F** and **G**) (from Povlishock and Kontos[85]).

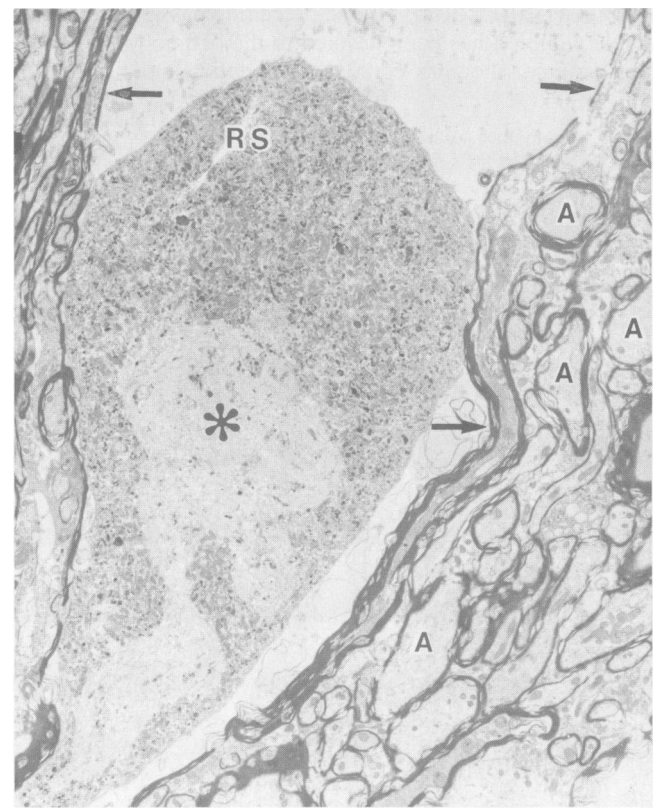

Figure 13.—The electron micrograph shows a reactive axonal swelling (**RS**) 24 hours after injury, with numerous organelles capping a neurofilamentous core (**asterisk**). A thinned, distended myelin sheath (**arrows**) surrounds the swelling. Despite the presence of this reactive axon, numerous surrounding axons (**A**) appear unremarkable (original magnification × 5,000).

tive, and sprouting axonal swellings were found (Figure 14). Degenerative and sprouting swellings now predominated, and these showed continued retrogressive or regenerative change, respectively. Many of the sprout-containing swellings showed continued differentiation, maturation, or both, with the number of total sprouts decreasing as the reactive axonal swelling now gave rise to growth cone-like processes. Such processes were 8 to 10 microns in diameter and up to 100 microns in length. These sprouts and growth cone-like processes frequently paralleled the sides of the overlying distended myelin sheath, and in those cases where the myelin investment was absent, these neuritic outgrowths were recognized to course into the substance of the surrounding unaltered brain-stem parenchyma.

With continued survival during the second and third months after trauma, the above-noted reactive changes were studied further. Both sprouts and growth cone-like processes were consistently found arising from the reactive axonal swellings, which, in general, were considerably reduced in mass as their volume was redirected into such outgrowing processes. In those animals studied during these survival periods, the reactive sprouting and overall regenerative processes showed considerable variation. Even well into the second month after injury, it was not uncommon to see newly sprouting profiles reminiscent of those seen in the early posttraumatic period. Apparently those swellings, which had persisted intact and unchanged during the early posttraumatic period, had now initiated a delayed form of regenerative response. The more mature sprouting and growth cone-containing swellings manifested further change. Overall, the number of sprouts per swelling was reduced. They were, however, longer and seemed to follow the overlying distended myelin sheath in a directed fashion (Figure 15). Such sprouts as well as the growth cone-like processes closely paralleled the distended myelin sheath and usually approximated defects in the myelin sheath, giving rise to the impression that they were entering the substance of the brain-stem parenchyma. In addition to those sprouts originating from the reactive swellings proper, other sprouts arose directly from the axonal shaft proximal to the swelling and from an adjacent node of Ranvier from where they gained easy access to the interstices of the brain parenchyma. We plan to analyze by serial sections the course and potential site of termination of all sprouting and growth cone-like processes.

The above-cited studies reaffirm our initial contention that reactive axonal change is a consistent feature of experimental head injury. Traumatically induced axonal swellings were consistently found, and it seemed that these persisted unchanged, degenerated, or initiated a regenerative response. The degree, manner, and duration of the observed regenerative response are remarkable and are a departure from classical thought regarding the potential for regeneration in the mammalian central nervous system. Although some investigators have advocated the concept of an initial postinjury regenerative response in the central nervous system, this response proved rapidly abortive and never

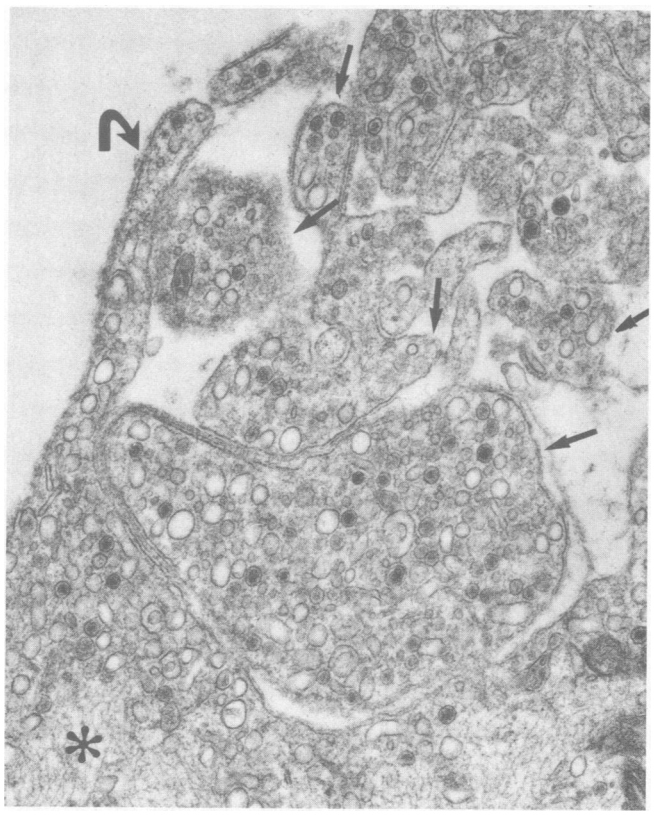

Figure 14.—The electron micrograph of reactive axonal changes three weeks after injury shows extensive sprouting at the tip of a swelling. One sprout (**curved arrow**) is in direct continuity with the swelling proper (**asterisk**). The other sprouts (**arrows**) in the field contain tubular and vesicular profiles of smooth endoplasmic reticulum and numerous dense core vesicles (original magnification × 30,000).

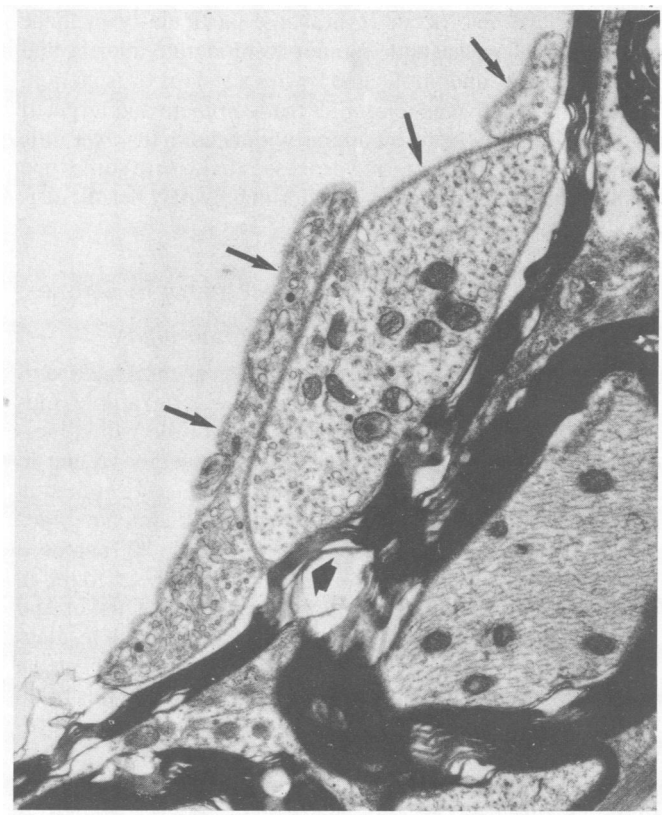

Figure 15.—The electron micrograph shows sprouts and growth cone-like processes (**long arrows**) seen in the second month after trauma. These processes parallel the distended myelin sheath overlying the swelling; defects in the myelin sheath (**block arrow**) suggest that they may serve as conduits for these processes to reach the surrounding brain parenchyma (original magnification × 16,000).

showed the sustained and continually evolving regenerative response seen in this study of head injury.[91,92]

The repertoire of regenerative responses described in the current investigation bears a striking similarity to those responses described in injured peripheral nerves and injured spinal cords of amphibians, who possess a significant regenerative capacity.[93,94] We can only speculate as to why head-injured cats showed such a sustained regenerative response. Perhaps some of those factors unique to minor-to-moderate head injury have contributed to this response. Because such injuries elicit axonal change with the retention of an otherwise unaltered brain parenchyma lacking significant gliosis or vascular insult, it seems conceivable that head injury may have created the ideal microenvironment for any potential regenerative response.

Much remains to be done on this issue. We do not know if with longer survival this regenerative attempt would continue and give rise to directed neuritic growth or rather abort, ending all hope for regeneration. Regardless of the long-term fate of this regenerative attempt, however, the finding of a sustained regenerative response with head injury must be considered intriguing. We may have unwittingly created a unique model for studying potential regeneration of the central nervous system and the effect of various therapeutic regimens.

The direct relevance of these animal studies to humans with head injury is somewhat uncertain. As reactive swellings are also a consistent feature of head injuries in humans, one would predict that changes comparable with those described above also occur in humans, particularly in those patients who have sustained minor-to-moderate injuries and whose course is uncomplicated by mass lesions or ischemia. We are not so naive as to predict that complete and targeted regeneration can occur in humans with head injury; yet, it is conceivable that regenerating processes may form some type of aberrant synaptic contacts that ultimately may permit neurologic improvement.

Biochemical Mechanisms of Cell Injury in Stroke

Glutamate Neurotoxicity in Ischemic Brain Injury

MARSHALL CHEUNG, PhD*: Evidence has accumulated suggesting that neuroexcitatory compounds—for example, glutamate and aspartate—play a major role in the pathophysiology of hypoxic-ischemic brain damage in in vivo and in vitro systems.[95-99] The pattern of cell damage in ischemia seems to coincide with areas of intense glutamate binding[100,101] and high-affinity glutamate uptake.[102,103] Increased neurotransmission mediated by glutamate has been found in the vulnerable regions of the hippocampus.[104] Diemer and co-workers reported that transient cerebral ischemia resulted in a 40% loss of hippocampal CA-1 neurons and suggested a possible neurotoxicity of the glutamate released in this brain region during ischemia.[105] In addition, these putative neurotransmitters have been implicated in the pathologic mechanisms associated with epileptic brain damage,[106] with Huntington's disease,[107,108] and with other neurodegenerative disorders—for example, olivopontocerebellar degeneration[109] and sulfite oxidase deficiency.[110] An elucidation of the role that glutamate and other excitatory neurotransmitters play in cellular injury is therefore important in our understanding of these and other similar brain disorders.

Convincing data are now available to implicate glutamate and possibly aspartate as excitatory transmitters in the mammalian nervous system.[111] They may be responsible for the fast excitatory postsynaptic potentials found in brain and spinal cord neurons.[104] Under normal conditions the extracellular glutamate concentration is kept low by sodium-dependent high-affinity uptake systems present in neurons and glia.[112-114] Under in vivo and in vitro ischemic conditions, however, a massive accumulation of brain extracellular glutamate and aspartate has been documented. The cellular origin of the released glutamate has recently been shown to be glutamatergic neurons.[115] In preliminary experiments, we showed a relatively specific fivefold increase in extracellular glutamate concentrations after in vitro incubation of rat retinas under anoxic conditions (Figure 16).[116] Under these conditions, serine levels increased only 1.5 times.

A similar release of glutamate has been shown in anoxic hippocampal neuronal cultures,[97] cerebellar slices,[96] and rabbit retina preparations.[95,117] With reperfusion or reoxy-

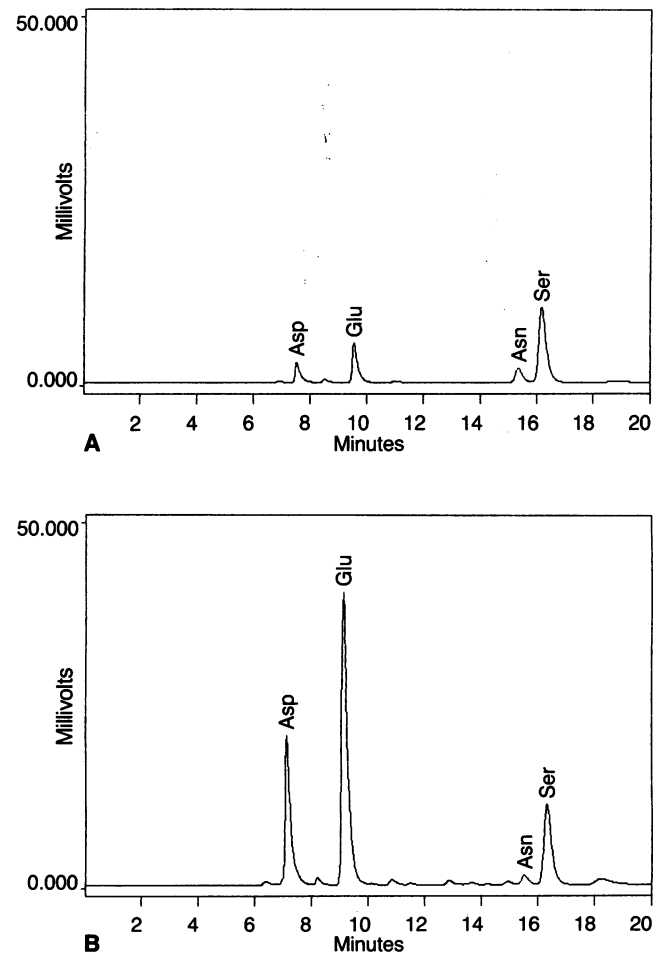

Figure 16.—The graphs show the release of glutamate from rat retina incubated in the presence (**A**) and absence (**B**) of oxygen. The released amino acids are identified by sampling the extracellular medium at 60 minutes. Each sample is collected and clarified before high-performance liquid chromatography analysis according to the protocol for physiologic amino acid analysis described by Jones and Gilligan.[116] Asn = asparagine, Asp = aspartate, Glu = glutamate, Ser = serine

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genation, or both, the increased levels of glutamate returned to normal,[95,117] but extracellular phosphoethanolamine concentrations continued to rise dramatically.[118] In addition, diglyceride levels containing stearate and arachidonate have also been shown to increase after ischemia.[118,119] Although direct evidence is still lacking, the increased levels of these compounds have been suggested to represent ischemia-induced membrane degradation. On the other hand, Dorman and associates[120] proposed an alternative pathway for the phosphoethanolamine liberation in which a loss of adenosine triphosphate during ischemia reverses reactions catalyzed by choline and ethanolamine phosphotransferase (EC 2.7.8.2 and EC 2.7.8.1, respectively) to liberate phosphocholine and phosphoethanolamine from their respective phospholipids.

Although the neurotoxicity of glutamate and aspartate has been known for 30 years, the mechanism of their action has remained elusive. Experimentally adding these neurotransmitters and other dicarboxylic amino acids has been shown to be neurotoxic in vivo and in vitro.[97,110,121] Under anoxic conditions, we found a biphasic effect of glutamate on rat retinal protein synthesis in an in vitro incubation system (Figure 17).[122-124] Stimulation of ^{3}H-phenylalanine incorporation was found at a glutamate concentration of 50 μmol, followed by a dose-dependent inhibition. When the rat retinas were incubated under normoxic conditions, adding glutamate stimulated protein synthesis at all concentrations tested.

Directly adding micromolar concentrations of glutamate also inhibited synaptosomal protein synthesis but only in the absence of magnesium ions (Figure 18).[125] Recent experiments in our laboratory, however, did not confirm the protective effect at 5 mmol Mg^{++}. Because Mg^{++}prevents the presynaptic release of glutamate[126] as well as inhibiting glutamate binding to its postsynaptic receptors,[122] the neurotoxic action of glutamate may be dependent on receptor interactions. The mechanism of cell injury following glutamate-receptor interactions is not known, however. The important relation between neurotransmitter-receptor interactions and membrane phospholipid breakdown (the phosphoinositide cycle) has been reemphasized[127-130] and may provide a mechanism for the neurotoxic action of glutamate. In partial agreement with this possibility, the inhibition of synaptosomal protein synthesis is partially reversed by 5 mmol lithium at higher glutamate concentrations (Figure 19). Lithium has been shown to disrupt the phosphoinositide cycle by inhibiting inositol-1-phosphate phosphatase, thereby preventing the cycling of inositol phosphates to inositol, which becomes rate limiting for the synthesis of membrane phosphatidylinositol.[130]

We hypothesize that, under ischemic conditions, there is a massive release of glutamate, which interacts with target neurons containing specific postsynaptic receptors for glutamate. Because glutamate receptors are not known to be down regulated[95] and sodium-dependent reuptake mechanisms are inactivated during conditions of ischemic energy deficit, there is uncontrolled and persistent neuronal stimulation by

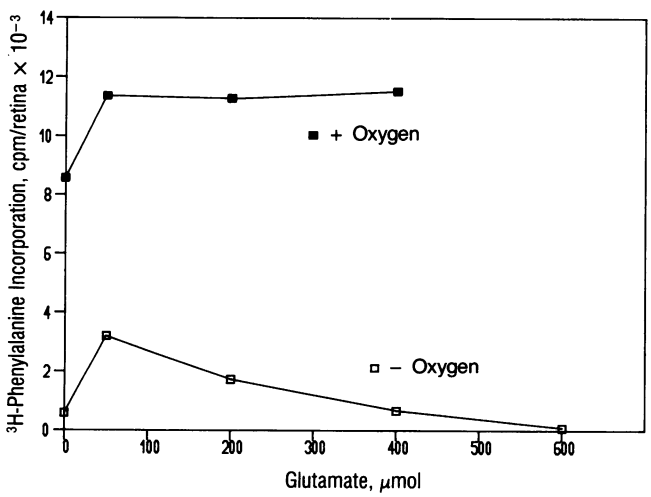

Figure 17.—The graph shows the in vitro effect of glutamate on the synthesis of proteins by rat retina. Rat retinas are incubated in an electrolyte solution described by Ames and Nesbett[122] in the presence and absence of oxygen and varying concentrations of glutamate. The rate of protein synthesis is estimated from the incorporation of ^{3}H-phenylalanine into trichloroacetic acid-insoluble material as described by Verity and colleagues[124] for synaptosomal protein synthesis.

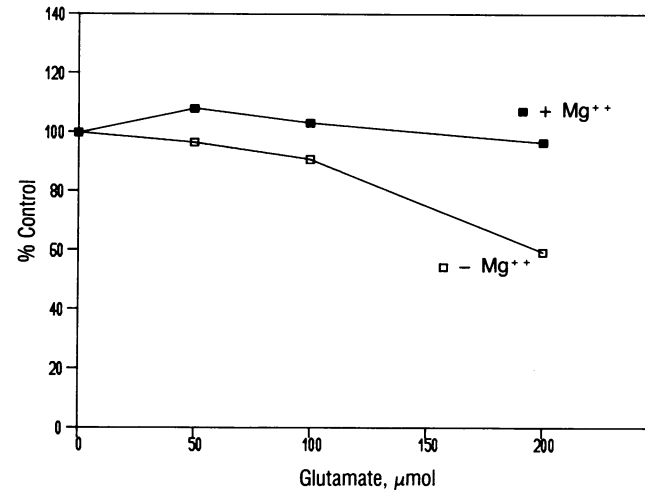

Figure 18.—The graph shows the effects of magnesium on glutamate perturbation of synaptosomal protein synthesis. Synaptosomes were prepared according to the method described by Verity and colleagues.[125] The effect of varying concentrations of glutamate on synaptosomal protein synthesis was monitored in the presence and absence of 10 mmol Mg^{++}.

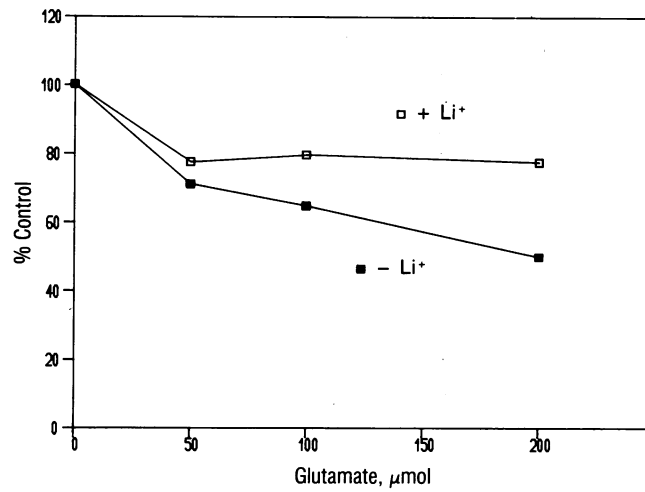

Figure 19.—The graph shows the protective effect of lithium on the glutamate perturbation of synaptosomal protein synthesis. Synaptosomes were prepared according to the method of Verity and colleagues[125] and incubated with varying concentrations of glutamate. In these experiments, incubations were done in a medium containing no Mg^{++} and 5 mmol lithium chloride.

continued glutamate-receptor interactions. Such interactions may abnormally activate the phosphoinositide cycle and perturb calcium homeostasis by stimulating extracellular calcium uptake and mobilizing intracellular calcium from the endoplasmic reticulum.[130] This increase in intracellular calcium levels then stimulates membrane degradation catalyzed by various calcium-dependent phospholipases, for example, phospholipase C and A_2. In addition to promoting membrane degradation and supplying arachidonic acid for the synthesis of eicosanoids, the phosphoinositide cycle stimulates the phosphorylation of a unique set of proteins through protein kinase C.

In cerebral cortex, it has been shown that cholinergic-muscarinic, α-adrenergic, and histamine H_1 receptors are linked to phosphoinositol breakdown. A similar link between glutamate receptor and the phosphoinositide-phosphoinositol cycle has yet to be shown (although recently a new class of glutamate receptor linked to inositol phospholipid metabolism has been reported[131]), nor has the possible role of abnormal activation of the phosphoinositide cycle in cell injury been explored. Brown and co-workers found that 1 mmol glutamate did not stimulate inositol phospholipid breakdown in rat brain cortical slices.[132] Our preliminary demonstration that lithium partially reversed the glutamate inhibition of protein synthesis in rat cortical synaptosomes suggests that such a link is possible but does not fully explain the glutamate effect. Work is in progress in our laboratory to determine the role of glutamate in ischemic cell injury and its relation to the phosphoinositide cycle, calcium mobilization, and membrane phospholipid breakdown.

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Traveler's Diarrhea—The Rule of Ps

FORTUNATELY, TRAVELER'S DIARRHEA IS A PREVENTABLE DISEASE. I recommend what I call the Traveler's Rule of Ps, that is, one should only consume foods and beverages that are peeled, packaged, purified, or piping hot. Most of the enteric organisms that cause diarrhea, as well as polio and hepatitis and so on, occur either in tap water, ice, or fresh fruits and vegetables.

If you can peel the fresh fruit and vegetables, they're safe. So mangos, oranges, bananas, and so on, are perfectly safe, no matter where you are. Grapes or tomatoes, however, unless you're willing to peel them, are not.

Packaged food is almost always safe. There have been occasional horror stories: bottled water in Mexico causing an epidemic of diarrhea, and so on, but this is very, very rare. In fact, if something seems reliably packaged and has a seal and looks like it was commercially done, then virtually everywhere in the world, it is safe to consume.

Purified refers primarily to water; and sterilized, purified water is now available, increasingly, throughout most of the common tourist destinations in Mexico and other parts of the commonly-visited developing world.

Remember, though, that ice can carry germs, too. This is one mistake that travelers make quite commonly. Unless the ice is purified—and it is very hard to get accurate information about this—it is best to stay away from it. Just because you're in a fancy place, a fancy hotel, does not mean that the ice is purified or that the water is safe to drink.

Piping hot is probably the most important thing. By that, I mean that if you eat food that has been cooked *now* and served piping hot *this moment* and not touched by anyone else between the time when it's hot and when you eat it, it is 95% + safe. Now, that's not to say something that was well cooked and has cooled off is safe.

—ROBERT B. BARON, MD

Extracted from *Audio-Digest Internal Medicine*, Vol. 34, No. 22, in the Audio-Digest Foundation's series of tape-recorded programs. For subscription information: 1577 E Chevy Chase Dr, Glendale, CA 91206